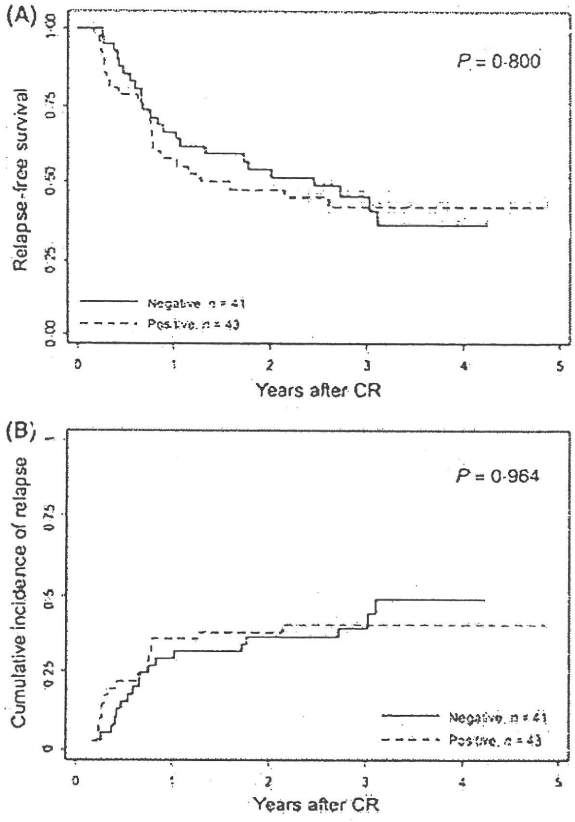

Fig 3. Relapse-free survival (A) and cumulative incidence of relapse (B) in patients with negative and positive *BCR-ABL1* transcript levels at the end of induction therapy.

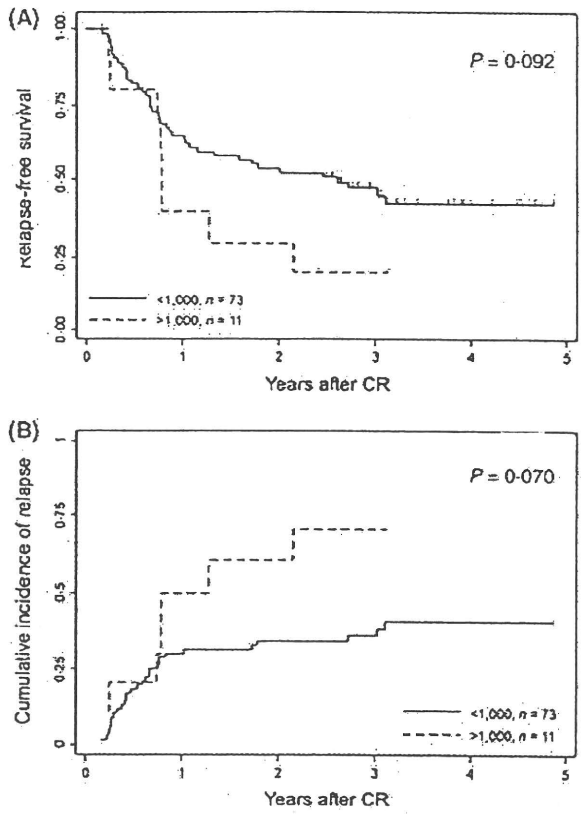

Fig 4. Relapse-free survival (A) and cumulative incidence of relapse (B) in patients with *BCR-ABL1* transcript levels below or above 1000 copies/μg RNA at the end of induction therapy.

MRD elevation during haematological remission

Elevated MRD levels during CR were documented in 29 patients. Of these, six patients experienced MRD elevation twice, and the second elevation was accompanied by simultaneous haematological relapse in five patients. The outcome and duration from the first observation of MRD elevation to relapse or allogeneic HSCT, whichever came first, in each patient are presented in Table II. Sixteen underwent allogeneic HSCT in first CR. The median duration from the first documentation of elevated MRD to allogeneic HSCT was 2·3 months (range 0·4–5·6). Death during first CR and relapse after transplantation occurred in three patients each, and 10 remained in first CR at a median of 2·9 years (range 2·0–4·6 months) after transplantation. In contrast, among the 13 non-transplantation patients, 12 had a relapse at a median of 2·0 months (range 0·5–35·0) after the first MRD elevation. Another patient once achieved PCR negativity after C#2, but showed detectable MRD below the threshold (<50 copies/μg) after C#5. However, MRD became negative after C#6, and the patient remained alive without relapse at 2·8 years after MRD elevation. The conversion from negative MRD to '<50 copies/μg' was observed in another six patients. Four remained in first CR after undergoing allogeneic HSCT, and the remaining two who had not undergone HSCT experienced a relapse.

Discussion

Minimal residual disease levels at various time points in CR, especially at the end of induction therapy, are considered an important prognostic factor in ALL (Pui *et al*, 2008). Although there were few studies that focused on Ph+ ALL with a relatively large number of patients (Dombret *et al*, 2002; Pane *et al*, 2005), Pane *et al* (2005) reported that significant reductions in *BCR-ABL1* levels after induction and consolidation therapy were associated with better outcomes. Most published studies on imatinib-combined chemotherapy include MRD findings (Thomas *et al*, 2004; Towatari *et al*, 2004; Lee *et al*, 2005; Rea *et al*, 2006; Wassmann *et al*, 2006; Yanada *et al*, 2006; de Labarthe *et al*, 2007; Ottmann *et al*, 2007a), but the prognostic significance of early treatment response remains to be determined. Our data remarkably demonstrated that the RFS rate for the patients with negative MRD at the end of induction therapy was similar to that for patients with positive MRD. We considered the possibility that this lack of difference was influenced by the confounding effect of allogeneic HSCT.

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Table II. Outcome of patients who experienced an MRD elevation during haematological CR.

UPN	Outcome	Months from MRD elevation to relapse	UPN	Outcome	Months from MRD elevation to HSCT	Outcome after HSCT
63	CCR without HSCT	–	36	HSCT	1·0	CCR
17	Relapse	0·5	72	HSCT	1·0	CCR
43	Relapse	0·9	12	HSCT	2·1	CCR
50	Relapse	1·5	77	HSCT	2·2	CCR
58	Relapse	1·5	10	HSCT	2·6	CCR
82	Relapse	1·6	1	HSCT	2·9	CCR
62	Relapse	1·9	81	HSCT	3·3	CCR
14	Relapse	2·0	94	HSCT	3·6	CCR
85	Relapse	3·6	55	HSCT	4·8	CCR
56	Relapse	4·3	8	HSCT	5·1	CCR
51	Relapse	7·9	34	HSCT	0·4	Relapse
60	Relapse	8·4	87	HSCT	2·0	Relapse
18	Relapse	35·0	47	HSCT	2·4	Relapse
			48	HSCT	1·9	NRM
			16	HSCT	2·0	NRM
			49	HSCT	5·6	NRM

UPN, unique patient number; MRD, minimal residual disease; HSCT, haematopoietic stem cell transplantation; CCR, continuous complete remission; NRM, non-relapse mortality.

However, MRD negativity was not beneficial in terms of relapse rate ($P = 0·964$) or even in terms of RFS after we censored patients who underwent allogeneic HSCT at the time of transplantation ($P = 0·470$). A trend toward a higher relapse rate in the 11 patients (13%) with MRD levels of ≥1000 copies/μg suggests that MRD levels at the end of induction therapy may be helpful in identifying a small subgroup of patients at high risk for relapse. However, the finding that negative MRD was not associated with a favourable outcome precludes prognostication of the remaining majority of patients, and indicates that relapse risk in these patients depends on factors unrelated to initial treatment response. Acquisition of resistance during treatment may explain why rapid molecular response is not prognostically relevant.

Another important finding of this study was the significant relationship between MRD elevation and relapse. This finding is in accordance with those of several studies published in the 1990s in which the conversion from negative to positive RT-PCR results was associated with subsequent relapse in Ph+ ALL patients (Miyamura *et al*, 1992; Preudhomme *et al*, 1997; Radich *et al*, 1997; Mitterbauer *et al*, 1999). Our results suggest that an increase in the MRD level at a single time point is predictive of subsequent relapse, but such patients can be successfully treated with allogeneic HSCT. Given a median duration of only 2 months from MRD elevation to haematological relapse, an alternative therapeutic intervention should be considered immediately after MRD elevation. Because of its rapid availability, cord blood transplantation may be a practical treatment option for patients without a related donor, if they are fit for the procedure. Switching from imatinib to other novel tyrosine kinase inhibitors, such as dasatinib (Talpaz *et al*, 2006; Ottmann *et al*, 2007b) and nilotinib (Kantarjian *et al*, 2006), may also be a reasonable option for patients without a mutation resistant to these agents. Additionally, frequent MRD monitoring increases the chances of detecting MRD elevation during CR, prolonging the duration prior to haematological relapse, and enabling the use of alternative therapies in patients who would otherwise experience an overt relapse.

When MRD data are analysed in relation to outcome, differences in conditions such as treatment and sampling time points can affect results. In this regard, strength of this study is that all samples were collected at scheduled time points during a uniform treatment protocol. On the other hand, one limitation of our study is that samples were not obtained from all patients at all time points. Nevertheless, the percentage of available samples collected at the end of induction therapy was 86% (84 of the 97 CR patients). Furthermore, sample availability did not seem to be a significant source of selection bias: we found no difference in RFS between patients whose samples were available or not at the end of induction therapy ($P = 0·345$). Also the utility of MRD elevation in predicting subsequent relapse would have been strengthened if the proportion of missing samples had been smaller. Finally, it may be disputed that our detection method was partly different from those used in other countries, specifically in that results were reported as a copy number normalized by the control gene and in that PCR negativity was not confirmed by nested PCR. Nevertheless, we believe this point would not impair our main results.

In summary, our prospective MRD monitoring of Ph+ ALL patients treated with imatinib-combined chemotherapy revealed that rapid molecular response is not associated with a superior prognosis and that a single observation of elevated

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MRD is strongly predictive of subsequent relapse but allogeneic HSCT can override its adverse effect. Such patients may also benefit from novel tyrosine kinase inhibitors. We conclude that frequent MRD monitoring is beneficial in clinical decision making for Ph+ ALL patients treated with imatinib-combined chemotherapy. Incorporating MRD data into a treatment protocol will be necessary in future clinical trials of Ph+ ALL.

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ORIGINAL ARTICLE

Diagnosis of acute myeloid leukemia according to the WHO classification in the Japan Adult Leukemia Study Group AML-97 protocol

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Abstract We reviewed and categorized 638 of 809 patients who were registered in the Japan Adult Leukemia Study Group acute myeloid leukemia (AML)-97 protocol using morphological means. Patients with the M3 subtype were excluded from the study group. According to the WHO classification, 171 patients (26.8%) had AML with recurrent genetic abnormalities, 133 (20.8%) had AML with multilineage dysplasia (MLD), 331 (51.9%) had AML not otherwise categorized, and 3 (0.5%) had acute leukemia of ambiguous lineage. The platelet count was higher and the rate of myeloperoxidase (MPO)-positive blasts was lower in AML with MLD than in the other WHO categories. The outcome was significantly better in patients with high (≥50%) than with low (<50%) ratios of MPO-positive blasts ($P < 0.01$). The 5-year survival rates for patients with favorable, intermediate, and adverse karyotypes were 63.4, 39.1, and 0.0%, respectively, and 35.5% for those with 11q23 abnormalities ($P < 0.0001$). Overall survival (OS) did not significantly differ between nine patients with $t(9;11)$ and 23 with other 11q23 abnormalities ($P = 0.22$). Our results confirmed that the cytogenetic profile, MLD phenotype, and MPO-positivity of blasts are associated with survival in patients with AML, and showed that each category had the characteristics of the WHO classification such as incidence, clinical features, and OS.

Keywords AML · WHO classification · Myeloperoxidase · Multilineage dyplasia · 11q23 abnormalities

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1 Introduction

The French-American-British (FAB) classification of acute myeloid leukemia (AML), based on morphological and cytochemical findings, was established in 1976 and has since become the standard classification [1, 2]. However, specific chromosomal and genetic abnormalities that have been extracted from analyses of prognostic factors for AML are recognized as important in selecting treatment strategies and are reflected in the AML classification as

factors that are required to establish the disease entity [3]. The 1999 World Health Organization (WHO) classification includes morphological, immunological, cytogenetic, genetic, and clinical features [4–6]. The WHO and FAB classifications differ in several aspects. The blast threshold required for a diagnosis of AML was reduced from 30 to 20%, and new AML categories have been added for cytogenetic abnormalities, the presence of multilineage dysplasia (MLD), as well as a history of chemotherapy and subtypes for acute basophilic leukemia, acute panmyelosis with myelofibrosis, and myeloid sarcoma. The WHO classification comprises more subtypes and is more comprehensive than the FAB classification.

Cytogenetic features are important prognostic factors in AML [3, 7–12]. However, 11q23 abnormalities have not yet been established as a cytogenetic risk classification. Over 30 partner genes with 11q23 abnormalities have been described, and some reports indicate that patients with $t(9;11)$ have a relatively more favorable prognosis than those with other partner chromosomes/partner genes [13–16].

In the present study, we reviewed stained smears of blood and bone marrow from patients who were registered in the Japan Adult Leukemia Study Group (JALSG) AML-97 trial, and classified them into FAB subtypes and WHO categories. We also evaluated their survival on the basis of the WHO classification, the myeloperoxidase (MPO)-positivity of blasts, and cytogenetic findings including 11q23 abnormalities.

2 Patients and methods

2.1 Patients

Between December 1997 and July 2001, 809 patients aged from 15 to 66 years with untreated AML (excluding M3) were registered from 103 institutions in the AML-97 trial of the JALSG. The patients were diagnosed with AML according to the FAB criteria at each institution. Patients with a history of MDS, hematological abnormalities before the diagnosis of AML, or a history of chemotherapy were not eligible for the AML-97 trial.

2.2 Treatment strategies

Details of the JALSG AML-97 treatment protocol are described elsewhere [17]. In brief, all patients underwent induction therapy consisting of idarubicin (3 days) and Ara-C (7 days). Patients who achieved complete remission were randomized into one of two arms of consolidation chemotherapy alone or in combination with maintenance chemotherapy. Patients who were placed into intermediate/poor risk groups according to the JALSG scoring system [17] and who had an HLA-identical sibling ($\leq$50 years old) were simultaneously assigned to receive allogeneic hematopoietic stem cell transplantation during their first remission.

2.3 Morphologic and cytochemical analyses

Peripheral blood and bone marrow smears from registered patients were sent to Nagasaki University for staining with May-Giemsa, MPO, and esterase, and the diagnosis was then reevaluated by the Central Review Committee for Morphological Diagnosis. Patients were subsequently categorized according to the FAB and WHO classifications. Dyserythropoietic features were defined as >50% dysplastic features in at least 25 erythroblasts and dysgranulopoietic features including $\geq$3 neutrophils with hyposegmented nuclei (pseudo-Pelger–Heut anomaly), and hypogranular or agranular neutrophils (>50% of $\geq$10 neutrophils). Dysmegakaryopoietic features were defined as $\geq$3 megakaryocytes that were micronuclear, multiseparate nuclear, or large mononuclear [18].

We assessed the ratios (%) of MPO-positive blasts on MPO-stained bone marrow smears using the diaminobenzidine method [19].

2.4 Cytogenetic analysis

Cytogenetic analysis was performed at either laboratories in participating hospitals or authorized commercial laboratories. The karyotypes of leukemic cells were collected through the JALSG AML-97 case report forms and reviewed by the Central Review Committee for Karyotyping. The patients were classified into favorable, intermediate, or adverse risk groups based on karyotypes according to results of the Medical Research Council (MRC) AML 10 trial [3]. The favorable risk group included patients with $t(8;21)$ and inv(16), whether alone or in combination with other abnormalities. The intermediate risk group included those with a normal karyotype and other abnormalities that were not classified as either favorable or adverse. The adverse risk group included patients with a complex karyotype with four or more numerical or structural aberrations, -5, deletion (5q), and -7, whether alone or in combination with intermediate risk or other adverse risk abnormalities.

2.5 Statistical analysis

The overall survival (OS) for all patients was defined as the interval from the date of diagnosis to that of death. We applied the Kaplan–Meier method to estimate OS and

Table 1 Patient characteristics

Age (year)	45 (15–66)
Male/female	390/248
WBC count ($\times 10^9$/l)	13.7 (0.4–709)
Hemoglobin (g/dl)	8.3 (3.8–17.2)
Platelet count ($\times 10^9$/l)	52 (0–890)
Bone marrow blasts (%)	56 (6–99)

Values are presented as the median (range)
WBC white blood cell

5-year survival. We compared survival rates between groups using the log-rank test (Stat View J 5.0). Differences were examined by the Chi-square test using Excel software. All *P*-values are two-sided, and values <0.05 were considered significant.

3 Results

3.1 Patient characteristics

Of the 809 registered patients, 638 were consistent with the WHO classification. Data were incomplete for 10 of the 638 patients. Table 1 lists the characteristics of the patients. The median age of all 638 patients (390 males and 248 females) was 45 years (range 15–66 years). The median values of WBC, hemoglobin (Hb), platelets, and the ratio of blasts in the bone marrow were 13.7 × 10^9/l, 8.3 g/dl, 52.0 × 10^9/l, and 56.0%, respectively.

3.2 FAB classification

Table 2 shows the FAB classification of the 638 patients. Most were classified as M2 ($n = 261$; 40.9%), followed by M4 ($n = 148$; 23.2%), and M1 ($n = 109$; 17.1%) with M0, M4Eo, M5a, M5b, M6, M7, and acute leukemia of ambiguous lineage comprising the remainder in that order.

3.3 WHO classification and clinical characteristics

Table 3 shows the patients categorized according to the WHO classification. The first category of AML with recurrent genetic abnormalities accounted for 171 patients (26.8%), 133 (20.8%) were in the second category of AML with MLD, 331 (51.9%) were in the fourth category of AML not otherwise categorized, and 3 (0.5%) were categorized as having acute leukemia of ambiguous lineage. Most patients in the second category were identical to those with a de novo MLD phenotype. We found that 144 patients diagnosed with the MLD phenotype comprised 133 (92.4%) in the second category, 10 (7.0%) with 11q23 abnormalities, and 1 (0.7%) with acute leukemia of ambiguous lineage. Figure 1 shows the OS of each category. The 5-year survival rates of the first, second, and fourth categories were 58.2, 22.5, and 40.9% ($P < 0.0001$), respectively.

Table 2 Number of patients according to the FAB classification

Subtype	Description	No. of patients	%
M0	Minimally differentiated acute myeloid leukemia (AML)	30	4.7
M1	AML without maturation	109	17.1
M2	AML with maturation	261	40.9
M4	Acute myelomonocytic leukemia (AMMoL)	148	23.2
M4Eo	AMMoL with eosinophils	23	3.6
M5a	Acute monoblastic leukemia	19	3.0
M5b	Acute monocytic leukemia	24	3.8
M6	Acute erythroleukemia	16	2.5
M7	Acute megakaryoblastic leukemia	5	0.8
Acute leukemia of ambiguous lineage		3	0.5
Total		638	100

Table 3 Number of patients according to the WHO classification

Category and subtype	No. of patients	%
I. AML with recurrent genetic abnormalities	171	26.8
t(8;21)(q22;q22);(AML1/ETO)	113	17.7
inv(16)(p13;q22) or *t*(16;16)(p13;q22);(CBFβ/MYH11)	26	4.1
t(15;17)(q22;q12)(PML/RARα)	–	–
11q23(MLL)abnormalities	32	5.0
II. AML with multilineage dysplasia	133	20.8
Following MDS	–	–
Without antecedent MDS	133	20.8
III. AML and MDS, therapy-related	–	–
Alkylating agent-related	–	–
Topoisomerase type II inhibitor-related	–	–
Other types	–	–
IV. AML not otherwise categorized	331	51.9
AML, minimally differentiated	25	3.9
AML without maturation	99	15.5
AML with maturation	108	16.9
Acute myelomonocytic leukemia (AMMoL)	63	9.9
AMMoL with eosinophilia	5	0.8
Acute monoblastic leukemia	8	1.3
Acute monocytic leukemia	16	2.5
Acute erythroid leukemia	6	0.9
Acute megakaryoblastic leukemia	1	0.2
Acute leukemia of ambiguous lineage	3	0.5
Total	638	100

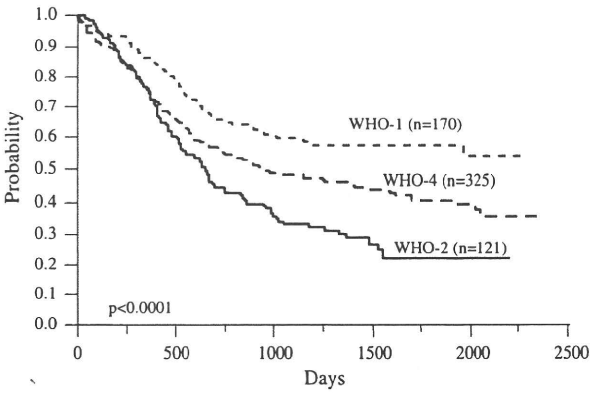

Fig. 1 Overall survival of patients categorized according to the WHO classification

Table 4 compares the clinical features among the WHO categories. The mean values of platelets, WBC, Hb, and the ratio (%) of blasts in bone marrow and of MPO-positive blasts significantly differed, whereas age did not significantly differ. Patients in the second category had a higher platelet count (111.0 × 10^9/l), whereas those with 11q23 abnormalities had a lower count (38.3 × 10^9/l) compared with those of other subtypes.

The WBC count of patients with *t*(8;21) was 1.4 × 10^9/l and lower than in other subtypes. The MPO-positive rate of blasts among patients with *t*(8;21) was higher (93.3%) and that of patients in the second category was lower (34.0%), than in other subtypes. All patients were grouped as high- or low-MPO according to ≥50% or <50% of MPO-positive blasts, respectively. A total of 339 patients (53.1%) were classified as high-MPO, 268 (42.0%) as low-MPO, and the MPO status of blasts could not be assessed in 31 (4.9%). Figure 2 shows the OS of patients with high- or low-MPO. The 5-year survival rate for patients with high or low-MPO was 50.7 and 29.6%, respectively ($P < 0.0001$).

3.4 Cytogenetics

All 638 patients were classified into favorable ($n = 139$; 21.8%), intermediate ($n = 413$; 64.7%), and adverse ($n = 54$; 8.5%) cytogenetic risk groups (Table 5). Figure 3 shows the OS according to this stratification. The 5-year survival rates were 63.4, 39.3, and 0.0% in the favorable, intermediate (except for those with 11q23 abnormalities), and adverse risk groups, respectively, and 35.5% in the group with 11q23 abnormalities ($P < 0.0001$).

The numbers of patients with or without MLD and high- or low-MPO in each cytogenetic risk group are listed in Table 6. None of those with the MLD phenotype were classified into the favorable risk group, while 129 (89.6%) and 15 (10.4%) of 144 patients with MLD were classified into intermediate or adverse risk groups, respectively. Only 15 patients (4.4%) in the high-MPO group were classified as having an adverse risk, while 11 (4.1%) in the low-MPO group were included in the favorable risk group.

The 32 patients with 11q23 abnormalities comprised 11 (34.4%) with *t*(11;19), 9 (28.1%) with *t*(9;11), 5 (15.6%) with del(11)(q23), 4 (12.5%) with *t*(6;11), and 3 (9.4%) with *t*(11;17). Figure 4 shows the OS of the intermediate risk group. The 5-year survival rate was 44.0% in patients with a normal karyotype, 35.5% in those with 11q23 abnormalities, and 30.6% in other patients including those with *t*(7;11), *t*(6;9), and Ph(+) abnormalities, respectively ($P = 0.033$).

Table 7 shows the relationship between *t*(9;11) ($n = 9$) and other 11q23 abnormalities ($n = 23$). More patients with low-MPO, without MLD, or with the FAB M5 subtype were found in the group with *t*(9;11) than with other 11q23 abnormalities. The survival rates between the two groups did not significantly differ ($P = 0.22$, data not shown).

4 Discussion

We attempted to classify selected patients who were reviewed morphologically and had available chromosomal data according to the WHO system. However, our series had some limitations in terms of analysis and patient selection. Although we obtained chromosomal data, genetic data were not available. Patients who were diagnosed with AML M3 or who had *t*(15;17), a history of MDS, or preceding hematological abnormalities, or who had previously undergone chemotherapy, were not eligible for the present study. However, multicenter trials might have some advantages in diagnosing AML according to the WHO classification, because morphological diagnoses and karyotypes are reviewed by the corresponding institutional committees.

The incidence of each category of the WHO classification was similar to those in several reports when patients with *t*(15;17) and therapy-related AML were excluded [20–22]. We and several others have shown that approximately 30% of patients have recurrent genetic abnormalities. Multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) assays have recently been applied to analyze cytogenetic abnormalities [21, 23, 24]. This method might cause the frequency of the first WHO category to increase. Thus, the multiplex RT-PCR assay might have to be incorporated into the WHO system. The JALSG has started a cohort study in which all AML patients in participating hospitals are registered and analyzed according to the WHO classification. That study should clarify the real ratios of the AML subtypes in the WHO classification.

Table 4 Comparison of clinical findings of patients diagnosed according to the WHO classification

Category		Platelets ($\times 10^9$/l ± SE)	WBC ($\times 10^9$/l ± SE)	Hb (g/dl ± SE)	Age (year ± SE)	Blasts in bone marrow (%±SE)	MPO positivity of blasts (%±SE)
I	t(8;21)	76.7 ± 56.43 (113)[a]	1.4 ± 0.6 (113)	7.8 ± 0.2 (113)	41.6 ± 1.3 (113)	49.9 ± 2.0 (113)	93.3 ± 3.3 (108)
	inv(16)	57.8 ± 52.03 (26)	6.6 ± 1.2 (26)	9.2 ± 0.5 (26)	44.5 ± 2.6 (26)	50.5 ± 4.1 (26)	66.9 ± 6.7 (26)
	11q23	38.3 ± 30.8 (32)	4.3 ± 1.1 (32)	8.9 ± 0.4 (32)	41.6 ± 2.4 (32)	56.3 ± 3.7 (32)	43.6 ± 6.1 (32)
II		111.0 ± 121.5 (133)	3.0 ± 0.5 (133)	8.3 ± 0.2 (133)	44.2 ± 1.2 (133)	48.0 ± 1.8 (133)	34.0 ± 3.1 (126)
IV		72.8 ± 91.7 (330)	5.1 ± 0.3 (331)	8.8 ± 0.1 (330)	43.8 ± 0.7 (331)	65.7 ± 1.2 (328)	53.7 ± 1.9 (312)
		$P < 0.0001$	$P < 0.0001$	$P = 0.0004$	$P = 0.4077$	$P < 0.0001$	$P < 0.0001$

SE standard error, *WBC* white blood cell, *MPO* myeloperoxidase, *Hb* hemoglobin

[a] Number of patients

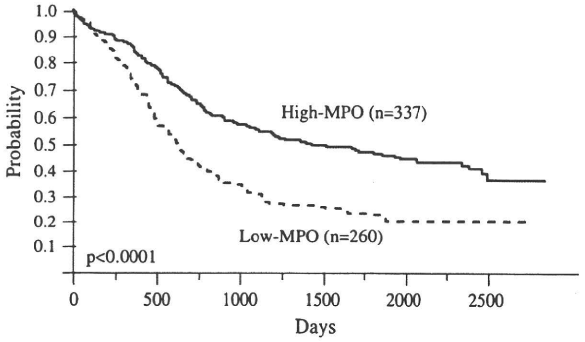

Fig. 2 Overall survival of patients with high or low MPO-positive blasts

Table 5 Distribution of patients classified by cytogenetic risk

Cytogenetic risk group	No. of patients	%
Favorable	139	21.8
t(8;21)	113	17.7
inv(16)	26	4.1
Intermediate	413	64.7
Normal karyotype	267	41.8
11q23	32	5.0
Ph(+)	7	1.1
t(7;11)(p15;p15)	4	0.6
t(6;9)	4	0.6
Other	131	20.5
Adverse	54	8.5
Complex	41	6.4
−7	2	0.3
abn3	5	0.8
del5q	2	0.3
−5	1	0.2
Other	3	0.5
Total	638	100.0

Few reports have included clinical data with the WHO classification. We found that the platelet count was higher among patients in the second category than in other categories. This supports our previous finding that the platelet count is higher in patients with AML accompanied by the MLD phenotype [25]. Among patients with MLD, none were in the favorable risk group, whereas the intermediate or adverse risk ratios among these patients were 89.6 and 10.4%, respectively. These differences might influence the finding that OS was better among patients without than with MLD ($P = 0.0002$, data not shown). Previous studies have also associated the MLD phenotype with a poorer outcome, although MLD is not significantly prognostic on multivariate analysis [18, 26], and a German group showed that dysplastic features correlate with adverse karyotypes

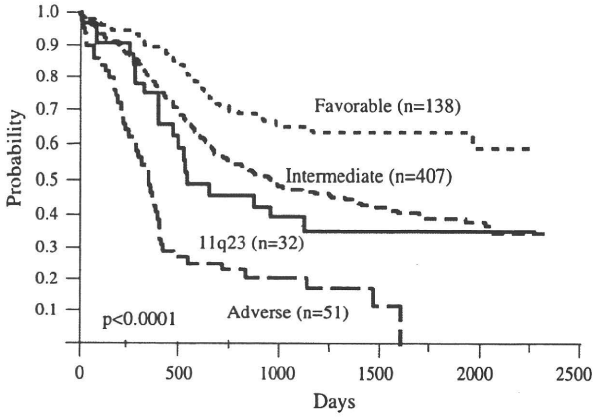

Fig. 3 Overall survival of patients stratified according to cytogenetic risk groups. Significant differences were observed between patients with a favorable, intermediate (except 11q23), and adverse karyotype ($P < 0.0001$)

Table 6 Relationship between cytogenetic risk groups and MLD phenotype or MPO-positive rates of blasts

	Favorable $n = 139$	Intermediate $n = 445$	Adverse $n = 54$	Total
MLD				
+	0	129 (89.5%)	15 (10.4%)	144
−	138 (28.2%)	292 (59.6%)	38 (7.8%)	490
Unknown	1	2	1	4
MPO				
High	123 (36.3%)	201 (59.3%)	15 (4.4%)	339
Low	11 (4.1%)	221 (82.5%)	36 (13.4%)	268
Unknown	5	23	3	31

High- and low-MPO indicates a percentage of myeloperoxidase positive blasts ≥50 or <50%, respectively

MLD multilineage dysplasia

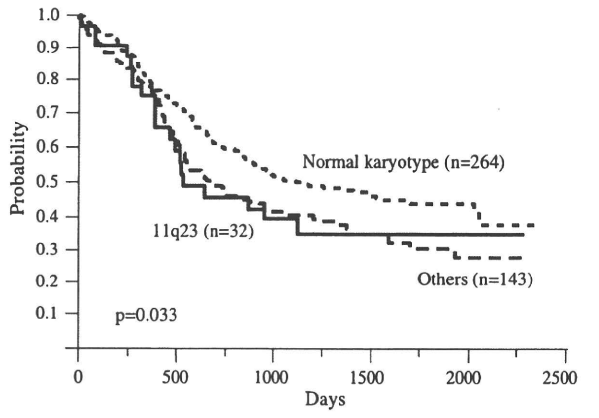

Fig. 4 Overall survival of patients with subtypes of intermediate cytogenetic risk. Significant differences were observed between patients with a normal karyotype and those with 11q23 abnormalities ($P = 0.033$)

[26]. Furthermore, patients in the second category had a lower MPO-positive rate of blasts, whereas those with $t(8;21)$ had a higher rate. Patients with high- and low-MPO were more frequently observed in the favorable and adverse risk groups, respectively. Multivariate analysis has shown that MPO is a significant factor affecting OS [19]. We did not assess prognostic factors by multivariate analysis here because the main theme of this study was to categorize patients according to the WHO classification, and we have already examined these in a previous series [18, 19].

Several studies have demonstrated the impact of specific cytogenetic abnormalities on survival in AML [3, 7–12, 20–22]. The cytogenetic risk groups stratified the AML patients in the present study according to the MRC system, as in these reports [3]. Therefore, we confirmed the clinical usefulness of cytogenetics as the first category of the WHO classification. We found that 32 patients had 11q23 abnormalities. The MRC system revealed that de novo and secondary AML patients with 11q23 abnormalities had an intermediate outcome with an OS rate of 45% at 5 years ($n = 60$; median age, 17 years) in a younger cohort [3] and an OS rate of 0% at 5 years ($n = 11$; median age 64 years) in an elderly cohort [7]. In contrast, SWOG/ECOG trials including adult de novo AML patients (age, 16–55 years) assigned those with 11q abnormalities to the unfavorable cytogenetic subgroup [8]. Our data showed that patients with 11q23 abnormalities have an intermediate rather than adverse outcome. The prognostic effect of 11q23 abnormalities might depend on the partner gene. Several studies have shown that 11q23 abnormalities with $t(6;11)$ and $t(10;11)$ are associated with a poor prognosis, whereas $t(9;11)$ is associated with a superior OS and such patients might respond well to intensive treatment, especially when the chemotherapy regimen includes high-dose cytarabine [15, 27–30]. The CALGB study has shown that the median OS of 13.2 months among 23 patients with $t(9;11)$ was significantly longer than the 7.7 months among 24 patients with other 11q23 rearrangements ($P = 0.009$) [30]. In a recent CALGB series of 54 patients with 11q23 abnormalities, 27 patients with $t(9;11)$ had an intermediate outcome and a median OS of 13.2 months, whereas those with $t(6;11)$ or $t(11;19)$ had a poor outcome of 7.2 or 8.4 months [15]. Conversely, Schoch et al. showed that 14 patients with $t(9;11)$ had a median OS of 10.0 months compared with the 12.8 months of 26 patients with other MLL rearrangements, and that the two cytogenetic groups did not significantly differ [13]. Our data showed that nine patients with $t(9;11)$ were more frequently involved in M5. The MPO and MLD features significantly differed between patients with $t(9;11)$ and those with other 11q23 abnormalities. However, the CALGB study found no significant differences in myelodysplastic features between the two

Table 7 Comparison of $t(9;11)$ and other 11q23 abnormalities

	No. of patients	Auer		MPO*		MLD*		FAB						Median age (year)	Median survival (day)
		+	−	High	Low	+	−	M1	M2	M4	M4Eo	M5a**	M5b		
$t(9;11)$	9	0	9	1	8	0	9	0	0	3	0	6	0	39	1031.00
Other 11q23	23	5	18	13	10	10	13	1	3	13	1	2	3	48	520.00
Total	32	5	27	14	18	10	22	1	3	16	1	8	3	44.5	531.5

High- and low-MPO indicates a percentage of myeloperoxidase-positive blasts ≥50 or <50%, respectively

MLD multilineage dysplasia

* $P < 0.05$, ** $P < 0.01$

cytogenetic groups [30]. In terms of OS, our results showed no significant differences between patients with $t(9;11)$ and those with other 11q23 abnormalities ($P = 0.22$). Some problems are associated with the analyses of 11q23 abnormalities. We had few patients with these abnormalities, particularly individual translocations, and genetic analysis was not performed. Thus, the prognostic risk of 11q23 abnormalities cannot be concluded from the present study. Nonetheless, these abnormalities were never associated with a favorable risk. To classify 11q23 abnormalities into each prognostic risk group, further investigations and genetic analyses of a large number of patients with 11q23 abnormalities are required.

The fourth WHO category, which is not otherwise categorized, accounted for 52% of patients in the present study. Most of them were classified into the intermediate risk group, and no prognostic subdivisions were valuable. Using cytogenetic features as a prognostic factor in groups with a normal karyotype has limitations, and such patients accounted for 64.6% of the intermediate risk group (data not shown). Additional factors are required to stratify these patients. We and several others suggested that differences could be based on molecular genetic analysis [22, 31–35]. For example, FLT3 mutations are important biomarkers of a normal karyotype and might be valuable for stratifying the intermediate risk group. Further follow-up studies might also shed light on the roles of FLT3 ITD mutations in the development of AML and aid their use as novel molecular targeting agents against AML [22, 32]. Bienz et al. identified CEBPA mutations, FLT3-ITD, and differing levels of BAALC expression as having independent prognostic significance in patients with a normal karyotype [33]. If these genetic markers can be confirmed as being of clinical significance, genetic analyses will probably be incorporated into the WHO classification.

In summary, our results confirmed those of previous studies showing the prognostic significance of cytogenetics, MLD, and MPO-positivity of blasts in AML. Furthermore, we categorized patients with de novo AML according to the WHO classification and showed the clinical characteristics and OS of each category.

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Karyotype at diagnosis is the major prognostic factor predicting relapse-free survival for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia treated with imatinib-combined chemotherapy

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ABSTRACT

To identify factors associated with relapse-free survival (RFS), 80 patients with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia, enrolled in a phase II study of imatinib-combined chemotherapy, were analyzed. The median follow-up of surviving patients was 26.7 months (maximum, 52.5 months). Twenty-eight out of 77 patients who had achieved CR relapsed. The probability of RFS was 50.5% at 2 years. Multivariate analysis revealed that the presence of secondary chromosome aberrations in addition to t(9;22) at diagnosis constitute an independent predictive value for RFS (p=0.027), and increase the risk of treatment failure by 2.8-fold.

Key words: acute lymphoblastic leukemia, Philadelphia chromosome, BCR-ABL, imatinib, karyotype.

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Introduction

The treatment for Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph$^+$ ALL) has changed dramatically since imatinib, a selective inhibitor of the ABL tyrosine kinase, was introduced.[1,2] Combined with chemotherapy, or even as a single agent, it can produce complete remission (CR) rates of 90% or higher in newly diagnosed patients.[3-9] We previously reported the results of a phase II study by the Japan Adult Leukemia Study Group (JALSG) to test the efficacy and feasibility of imatinib-combined chemotherapy for newly diagnosed Ph$^+$ ALL.[6] The rate of CR reached 96%, and that of BCR-ABL negativity in bone marrow 71%. However, despite a relatively short follow-up period, relapse occurred in a subset of the patients who had achieved CR.

On the other hand, remarkable progress is being made with the development of novel tyrosine kinase inhibitors with more potent *in vitro* and *in vivo* activities than imatinib.[10,11] Given this, we investigated factors associated with relapse-free survival (RFS).

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Design and Methods

Patients and treatments

Eligibility criteria included newly diagnosed Ph' ALL, age between 15 and 64 years, an Eastern Co-operative Oncology Group performance status between 0 and 3, and adequate liver, kidney and heart function. Written informed consent was obtained from all patients prior to registration.

For remission induction therapy, imatinib was administered from day 8 to day 63 in combination with daunorubicin, cyclophosphamide, vincristine (VCR) and prednisolone (PSL). Consolidation therapy consisted of an odd course (C1) comprising high-dose methotrexate, high-dose cytarabine and methylprednisolone, and an even course (C2) with single-agent imatinib for 28 days. C1 and C2 were alternated for 4 cycles each. After completion of the consolidation therapy, patients received maintenance therapy consisting of VCR, PSL and imatinib for up to 2 years from the date CR had been achieved.[6] The daily dose of imatinib used in this study was 600 mg. Allogeneic hematopoietic stem cell transplantation (HSCT) was recommended if a matched sibling donor was available, and was allowed from an alternative donor.

The protocol was reviewed and approved by the institutional review board of each of the participating centers and was conducted in accordance with the Declaration of Helsinki.

Cytogenetic and molecular analysis

At diagnosis, bone marrow samples were examined for cytogenetic abnormalities with standard banding techniques. Karyotypes were classified according to the International System for Human Cytogenetic Nomenclature.[12] The number of BCR-ABL copies in bone marrow was determined at a central laboratory with the real-time quantitative RT-PCR test according to the previously described method.[13]

Statistical analysis

Kaplan-Meier survival analysis was performed to estimate the probabilities of RFS, event-free survival (EFS), and overall survival (OS), with differences between the groups compared by the log-rank test. Cumulative incidences of relapse were calculated with non-relapse mortality considered as a competing risk, and differences between the groups were compared with the Gray's test. For risk factor analysis, a Cox proportional hazards model was constructed. In multivariate analysis, variables with p values of <0.10 determined by univariate analysis were included in the final model. A hazard ratio (HR) was calculated in conjunction with a 95% confidence interval (CI).

Results and Discussion

A total of 80 patients were recruited between September 2002 and January 2005. The median age was 48 years (range 15-63), with 49 males and 31 females. CR was achieved by 77 (96.2%) patients. During a median follow-up of 26.7 months (maximum 52.5 months), 28 patients relapsed. Of the 17 relapses observed during the consolidation therapy, 13 occurred during the imatinib course. The probabilities of EFS and OS were 48.5±5.7% and 58.1±5.7% at 2 years (Figure 1). For patients who had achieved CR, the probability of RFS was 50.5±5.9% at 2 years. Allogeneic HSCT was performed for 60 patients, including 24 from a sibling donor, 1 from a related donor other than a sibling, 25 from an unrelated donor, and 10 from unrelated cord blood. Disease status at the time of transplantation was first CR for 44 patients, second CR for 4 and non-CR for 12. The 2-year RFS for those who had undergone allogeneic HSCT during first CR was 62.6±7.5% and 62.1±12.3% for those who had not undergone allogeneic HSCT. When allogeneic HSCT was considered as a time-dependent covariate, it was shown to have no significant effect on RFS (HR, 1.03; 95% CI, 0.51-2.09; p=0.934). Major and minor BCR-ABLs were detected in 23 and 56 patients respectively. The transcript type of the remaining patient could not be determined because fluorescent *in situ* hybridization analysis was used instead of the PCR test. Neither transcript types nor copy numbers at diagnosis were associated with RFS (p=0.763 and 0.912). Pre-treatment cytogenetic results were not available for 4 patients because analysis was not performed (n=2) or was not successful (n=2). Of the remaining 76 patients, 22 showed only t(9;22) or variant translocations, 51 showed additional chromosome aberrations, and 3 showed normal karyotype. Additional aberrations exceeding a frequency of 10% comprised +der(22)t(9;22) in 17 patients, abnormalities involving the short arm of chromosome 9 [abn(9p)] in 17, monosomy 7 in 10, and trisomy 8 in 10. Figure 2 compares RFS for patients with and without additional chromosome aberrations. The presence of additional aberrations was significantly associated with shorter RFS (p=0.003). The relapse rate was also higher in patients with additional aberrations (41% *vs.* 20% at 2 years, p=0.0414). Analyses of the 4 recurrent abnormalities mentioned above demonstrated a statistically significant negative impact on RFS for +der(22)t(9;22) and abn(9p) (p<0.001 and p=0.005). Even after allogeneic HSCT, patients with additional aberrations appeared to have a trend for shorter RFS than those without (p=0.080), but this might reflect a larger proportion of transplantation beyond first CR in the former (31% *vs.* 17%). In patients allografted during first CR, there was no difference in cumulative incidences of relapse dated from the day of transplantation between the 2 groups

(16.5% *vs.* 12.5% at 2 years, p=0.546). Variables that showed a significant effect on RFS in the univariate Cox model included additional chromosome aberrations (p=0.005), peripheral blood blasts % (p=0.024) and sex (p=0.03). Results of multivariate analysis are shown in Table 1. The presence of additional chromosome aberrations was identified as the only independent prognostic factor for RFS (p=0.027). These updated data strongly support recent reports showing the feasibility and remarkable efficacy of imatinib-combined chemotherapy for newly diagnosed Ph+ ALL.[3-9,14,15] The main objective of this report was to identify factors affecting RFS, an issue of rapidly increasing importance given the development of novel tyrosine kinase inhibitors which are expected to further expand the treatment options for this disease. Our data indicated that additional chromosome aberrations, particularly +der(22)t(9;22) and abn(9p), were associated with shorter RFS. It is well known that additional chromosome aberrations are seen frequently in Ph+ ALL. Before the imatinib era, some groups reported the prognostic relevance of additional aberrations.[16-18] By contrast, from a large series of 204 patients, Moorman *et al.*[19] recently showed no significant effect of specific additional aberrations, including +der(22)t(9;22) and del(9p), on survival. In this study, analyzing patients treated with imatinib-combined chemotherapy, the 2-year RFS rate exceeded 80% for those without additional aberrations, whereas outcomes for those with additional aberrations were relatively unfavorable.

Acquisition of resistance to imatinib is an emerging problem in the treatment of chronic myeloid leukemia. One of the most common mechanisms of resistance is the mutation involving the ABL kinase domain. Although it has not been confirmed whether such mutations compromise the clinical outcome of Ph+ ALL patients treated with imatinib-combined chemotherapy, our observation that most of the early relapses occurred during the consolidation courses consisting of imatinib alone implies possible imatinib resistance. If that is the case, switching from imatinib to other novel tyrosine kinase inhibitors based on the pre-treatment cytogenetic results soon after achieving CR or even earlier could be an alternative treatment approach for further improving outcome in Ph+ ALL. Lack of mutation analysis is a major limitation of this study. Recently, Pfeifer *et al.*[20] studied the ABL kinase domain mutation status in newly diagnosed Ph+ ALL patients who were treated with imatinib-combined chemotherapy, and showed that even before exposure to imatinib, mutations were detected in 38% of patients. Importantly, the frequency of the mutant allele was low in such patients. However, at the time of relapse, the same mutation was present as the dominant clone in 90% of the relapsing cases.[20] Altogether, further insights will be provided by investigating the association between karyotype and mutation status at diagnosis.

Despite such limitations, the analysis of 80 patients entered into a single trial identified karyotype at diagnosis as a significant prognostic factor for RFS in newly diagnosed Ph+ ALL patients treated with imatinib-combined chemotherapy. Although our results need to be confirmed regarding kinase domain mutation status, these findings may play a critical role in the future treatment of Ph+ ALL.

Table 1. Multivariate analysis of factors associated with relapse-free survival.

P-value	*HR (95% CI)**	*Factors*	
Additional [chromosome aberrations	0.027	2.84 (1.12-7.19) 1.00	Present Absent
Peripheral blood blasts%	0.051	1.12 (1.00-1.22)	Per 10% increase
Sex	0.148 1.00	1.73 (0.82-3.64) Female	Male

*HR, hazard ratio; 95% CI, 95% confidence interval. *Values higher than unity indicate higher risk for failure.*

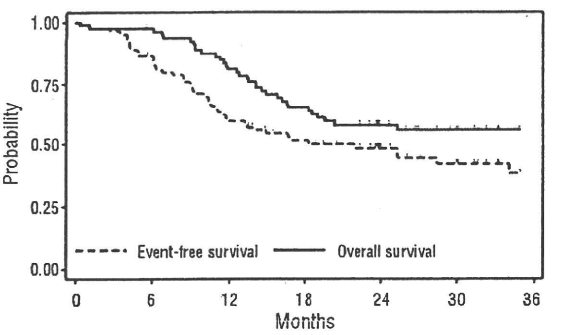

Figure 1. Kaplan-Meier curves for event-free and overall survival. The probabilities of event-free and overall survival at 2 years were 48.5% and 58.1% respectively (n=80).

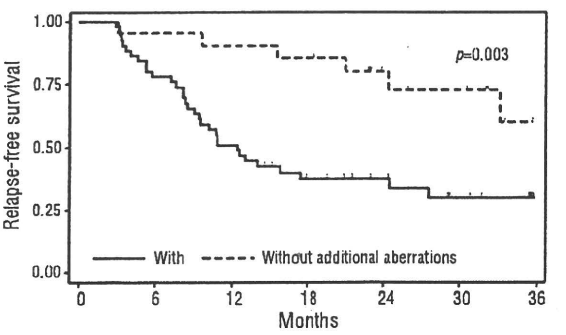

Figure 2. Relapse-free survival for patients with and without additional cytogenetic aberrations. Patients with additional cytogenetic aberrations (n=50) had significantly shorter relapse-free survival than those without (n=20).

Authorship and Disclosures

MY designed and co-ordinated the study, analyzed the data, and wrote the paper; JT, NU, FY, SM, and IJ designed the study, and provided patient sample and clinical data; IS, HA, KN, YU, MT, and AM provided patient sample and clinical data; HN co-ordinated the study, and revised the paper. YM provided patient sample and clinical data and engaged in data management. SO designed the study, provided patient sample and clinical data, and engaged in data management; KM designed the study, and analyzed the data; TN chaired the study group, co-ordinated the study, and revised the paper; RO served as the principal investigator, chaired the study group, and revised the paper. All authors reviewed the paper, interpreted the results, and approved the final version. The authors reported no potential conflicts of interest.

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